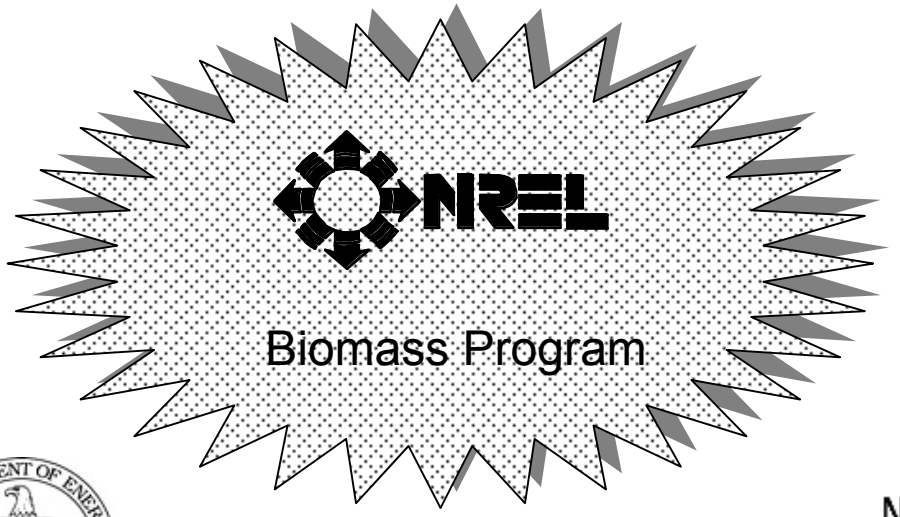

Biomass Analysis Technology Team
Laboratory Analytical Procedure

DRAFT
Version 2004

Procedure Title:
Preparation of Samples for Compositional Analysis

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1. Introduction

1.1 This procedure describes a reproducible way to convert a variety of biomass samples into a uniform material suitable for compositional analysis. The National Renewable Energy Laboratory (NREL) LAPs for compositional analysis have been optimized using samples with a specific particle size range and moisture content. All NREL biomass compositional analysis procedures assume that the samples have been prepared to meet these specifications. Deviations from these parameters may invalidate assumptions made in those methods and thereby introduce errors in the analysis. Procedures are listed that are suitable for the preparation of biomass feedstocks and a variety of biomass-derived materials. Representative sampling of biomass samples is also addressed.

1.2 This procedure is substantially similar to ASTM Standard Practice E 1757 – 01. Parts of this procedure are similar to TAPPI method number T264 and NFTA method A 1.1.

1.3 This procedure describes methods for drying, size reduction, obtaining samples with a uniform particle size and representative sampling of biomass samples.

2. Scope

2.1 This method is appropriate for the preparation of most types of biomass and biomass-derived solids for compositional analysis.

2.2 This procedure is not intended for materials that will already pass through a 20-mesh sieve.

2.3 This procedure is not intended for materials that cannot be dried by the described methods to a total solids content of greater than 85% of the sample's oven dried weight.

2.4 All analyses shall be performed according to the guidelines established in an appropriate Quality Assurance Plan (QAP).

3. Terminology

3.1 *Ambient conditions*: a temperature of 20°C to 30°C (68°F to 85°F), less than 50% relative humidity.

3.2 *Prepared biomass*: biomass that has been prepared according to this practice.

3.3 *Oven dry weight (ODW)*- the weight of biomass mathematically corrected for the amount of moisture present in the sample at the time of weighing

4. Significance and Use

4.1 This procedure is used, in conjunction with other procedures to determine the chemical composition of biomass samples, see LAP "Summative Mass Closure for Biomass Samples".

4.2 This procedure describes drying, size reduction and representative sampling methods that must be performed prior to analysis for many other constituents.

5. Interferences

5.1 This procedure produces biomass samples with a particle size range. The NREL procedures for biomass compositional analysis have been optimized for samples with this particle size range. Deviation to a smaller particle size may result in a low bias in carbohydrate content (and consequent high lignin bias) due to excessive carbohydrate degradation. Deviation to a larger particle size may also result in a low bias in carbohydrate content (and consequent high lignin bias) due to incomplete hydrolysis of polymeric sugars to monomeric sugars.

5.2 This procedure produces samples with moisture contents below 10%. The NREL procedures for biomass compositional analysis have been optimized for samples with low moisture contents. Higher moisture content in biomass samples will alter the effective acid concentration in the concentrated acid hydrolysis steps. Lowering the acid concentration may result in a low bias in carbohydrate content due to incomplete hydrolysis of polymeric sugars to monomeric sugars. Incomplete hydrolysis leaves many oligomeric sugars, which are not soluble in 4% acid and are incorrectly counted as acid insoluble residue in these procedures, introducing a consequent high lignin bias.

5.3 This procedure assumes that chemical fractionation does not occur during the sieving steps and that the chemical composition of any fines removed from the sample is substantially similar to the composition of the bulk sample.

6. Apparatus

6.1 Large table or drying rack for air drying biomass (method A only).

6.2 Convection oven capable of maintaining 45 ±5ºC (method B only).

6.3 Freeze-Drier - System with vacuum chamber and pump capable of maintaining a pressure of <1 torr and a cold finger in the chamber capable of maintaining a temperature of -50°C (method C only).

6.4 Balance, sensitive to 0.1 g.

6.5 Standard laboratory knife mill with 2 mm screen. A Wiley Mill, size No. 4 with a 2-mm screen, is suitable for samples >20 g, and the intermediate model Wiley Mill, with 1-mm screen, is suitable for samples <20 g that will not be sieved. Equivalent knife mills are acceptable.

6.6 Sieve Shaker that provides motion in both horizontal and vertical axes.

6.7 Sieve Set, No. 20 (850 µm), No. 80 (180 µm) stackable sieves with lid and bottom pan. Sieves and bottom pan should be 8.9 cm (31/2 in.) in height. Sieves should conform to ASTM Specification E 11.

6.8 Riffle Sampler with Pans— A manual sample divider that splits the milled biomass into two or more equivalent sub-samples. Riffle divisions should be between 6.4 mm and 12.7 mm (1/4 to 1/2 in.) with at least twenty-four riffle openings. The feed chute and riffles should have a slope of at least 60°. Collection pans, one to pour the sample into the riffler, and two or more to collect the sub–samples.

7. Reagents and materials

7.1 Reagents

7.1.1 Acetone, electronic grade.

7.1.2 Dry ice, ground.

7.2 Materials

7.2.1 Assorted trays and containers as appropriate for the selected drying method.

8. ES&H Considerations and Hazards

8.1 Milling and sieving actions both produce large amounts of dust. This dust can be a nuisance, hazard and irritant. Use appropriate respiratory protection and eye protection as needed.

8.2 If excessive amounts of dust are allowed to become airborne, a potential explosion hazard is possible. Provide appropriate dust control measures as needed.

8.3 Follow all applicable NREL chemical handling procedures.

8.4 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

9. Sampling, Test Specimens and Test Units

9.1 This procedure describes sampling, and sample preparation required prior to analysis for ash, extractives, carbohydrates, lignin, and protein.

10. Procedure

10.1 Prepare the biomass sample using one of the three methods described below.

10.2 Sample Preparation Method A: Air-drying. This method is suitable for the preparation of large quantities (>20 g) of field-collected samples into a form appropriate for compositional analysis. This method is suitable for drying materials where ambient humidity allows the sample to air-dry to a moisture content below 10% as measured using LAP "Determination of Total Solids in Biomass" (2004).

10.2.1 Biomass samples must first be available as pieces with overall dimensions less than 5 by 5 by 0.6 cm (2 by 2 by 1/4 in.). Stems or twigs should not exceed 0.6 cm (1/4 in.) diameter. It is recommended that wastepaper should be shredded into pieces less then 1 cm (3/8 in.) wide. Twigs, straw and wastepaper should not exceed 20 cm (8 in.) in length to facilitate milling.

10.2.2 The biomass material should be spread out on a suitable surface and allowed to air-dry prior to any milling. Do not pile the material deeper than 15 cm. Turn the material at least once per day to ensure even drying and Microbial growth in samples.

10.2.3 Following NREL "LAP Determination of Total Solids in Biomass" (2004) measure the solids content of the biomass sample once every 24 hours.

10.2.4 The material is considered dried when the moisture content is less than 10% and the change in weight is less than 1% in 24 h.

10.2.5 Feed the air-dried biomass into the knife-mill and mill until the entire sample passes through the 2 mm screen in the bottom of the mill. Laboratory mills can generate enough heat to damage biomass samples. Monitor the mill closely and allow the mill to cool to room temperature between batches if necessary.

10.2.6 Stack the sieves in the following order, starting at the bottom: the bottom pan, 80-mesh sieve, 20-mesh sieve.

10.2.7 Place the milled biomass in the 20-mesh sieve. The sample should be no more than 7 cm deep in the 20-mesh sieve. The milled sample may be processed in batches if necessary.

10.2.8 Place the cover on the sieve stack and secure the stack in the sieve shaker.

10.2.9 Shake the sieves for 15 ± 1 min.

10.2.10 The fraction retained on the 20-mesh sieve (+20 mesh fraction) should be reprocessed (steps 10.2.5 through 10.2.9) until no biomass remains on the 20-mesh sieve.

10.2.11 The fraction retained on the 80-mesh sieve (-20/+80 mesh fraction) should be retained for compositional analysis.

10.2.12 The material in the bottom pan is the fines (-80mesh) fraction. Retain this material for ash analysis.

10.2.13 Combine all of the -20/+80 mesh batches. Weigh the combined -20/+80 mesh fraction to the nearest 0.1 g. Record the weight of the -20/+80 mesh fraction as $Wt_{20/80}$.

10.2.14 Combine all of -80 mesh batches. Weigh the combined fines to the nearest 0.1 g. Record the weight of fines fraction as Wt_{80}.

10.2.15 If multiple sieved samples were combined they must homogenized. Pour the -20/+80 mesh fraction into the riffle sampler.

10.2.15.1 The sample must be distributed evenly onto all the riffle openings. A pan, as wide as the riffle opening, should be used. Pour the sample evenly off the entire side of the pan and not from the end or the corner. Do not transfer the biomass sample from a narrow-mouth container such as a jar.

10.2.16 Recombine the riffled sub-samples.

10.2.17 Repeat steps 10.2.15 through 10.2.16 a total of four times

10.2.18 Determine the total solids content (TS) of both the –20/+80 mesh fraction and the fines fraction using LAP "Determination of Total Solids in Biomass" (2004). Record the total solids of the -20/+80 mesh fraction as $TS_{20/80}$. Record the total solids content of the fines as TS_{-80}.

10.2.19 Determine the ash content of each fraction using LAP "Determination of Ash in Biomass" (2004). Record the ash content of the -20/+80 mesh fraction as $Ash_{20/80}$. Record the ash content of the fines as Ash_{-80}.

10.2.20 Using the equations in Section 11, calculate the percent of each fraction in the original, biomass sample. The fraction weight percent is used to reconstruct the composition of the original biomass sample. In most cases, the composition of non-ash biomass in the fines can be assumed to be the same as the composition of the non-ash biomass in the –20/+80 fraction.

10.2.21 If the total sample needs to be subdivided into smaller samples, use the riffler to make this separation.

10.2.22 If the prepared sample is not analyzed immediately after sieving and riffling, the sample should be stored in an airtight container or sealable polyethylene bag and kept at -20°C until needed.

10.3 Sample Preparation Method B: Convection oven drying. An acceptable alternative to air-drying is to dry the biomass sample in a convection oven at temperatures no greater than 45°C. This method is suitable for very wet biomass that is at risk for microbial growth during drying, wet pretreated biomass, samples that would not be stable during prolonged exposure to ambient conditions, or for drying materials when ambient humidity does not allow the sample to air-dry to a moisture content below 10% as measured using LAP "Determination of Total Solids in Biomass" (2004). This drying method is suitable for small samples of biomass (<20 g).

10.3.1 Select a container suitable for oven drying the biomass sample and dry this container at 45± 3°C for a minimum of 3 h.
10.3.2 Place the container in a dessicator and allow the container to cool to room temperature.
10.3.3 Weigh the container to the nearest 0.1 g and record this weight as Wt.
10.3.4 Place the biomass material into the dried container to a maximum depth of 1 cm.
10.3.5 Weigh the container and biomass to the nearest 0.1 g and record this weight as W_i.
10.3.6 Place the container and biomass in a drying oven maintaining the temperature at 45 ± 3°C. Allow the material to dry for 24 to 48 h.
10.3.7 Remove the container and biomass from the drying oven, place in a desiccator and allow the sample to cool to room temperature.
10.3.8 Weigh the container and biomass to the nearest 0.1 g and record this weight as W_f.
10.3.9 Return the sample to the drying oven, maintaining the temperature at 45 ± 3°. Keep the sample in the drying oven at 45 ± 3°C for minimum of 4 h .
10.3.10 Remove the container and biomass from the drying oven, place in a desiccator and allow the sample to cool to room temperature
10.3.11 Weigh each sample to the nearest 0.1 mg and record this weight.
10.3.12 Return the samples to the drying oven at 45°C for 1 h.
10.3.13 Remove the container and biomass from the drying oven, place in a desiccator and allow the sample to cool to room temperature.
10.3.14 Weigh each sample to the nearest 0.1 mg and record this weight.
10.3.15 Repeat steps 10.3.12 through 10.3.14 until the change in the mass of the biomass is less than 1%.
10.3.16 Mill the dried biomass sample using one of the following procedures.
10.3.16.1 For small quantities (<20 g) containing material that would not pass through a 20 mesh screen, reduce the particle size of the solid residue by knife-milling the entire sample through an intermediate size knife-mill with a 1 mm screen. If the sample size is sufficient, sieve the sample as described in Method A, steps 10.2.5 through 10.2.14.
10.3.16.2 For larger quantities (>20 g) containing material that would not pass through a 20 mesh screen, reduce the particle size of the solids by knife-milling, sieving and riffling the entire sample as described in Method A steps 10.2.5 through 10.2.18.
10.3.17 Using the equations in Section 11, calculate the percent of each fraction in the original biomass sample. The mass fraction is used to reconstruct the composition of the original biomass sample. In most cases, the composition of non-ash biomass in the fines can be assumed to be the same as the composition of the non-ash biomass in the –20/+80 fraction.
10.3.18 If the total sample needs to be subdivided into smaller samples, use the riffler to make this separation whenever possible.
10.3.19 If the prepared sample is not analyzed immediately after sieving and riffling, the sample should be stored in an airtight container or sealable polyethylene bag and kept at -20°C until needed.

10.4 Sample Preparation Method C: Lyophilization. An acceptable alternative to air-drying (Method A) or drying in a convection oven (Method B) is lyophilization (freeze-drying) of the sample. This method is suitable for very wet biomass that is at risk for microbial growth during drying, wet pretreated biomass, samples that would not be stable during prolonged exposure to ambient conditions, or for drying materials when ambient humidity does not allow the sample to air-dry to a moisture content below 10% as measured using LAP "Determination of Total Solids in Biomass" (2004). This test method is also suitable for materials that are heat sensitive and would degrade if subjected to the drying oven in Test Method B. This drying method is suitable for small samples of biomass (<20 g).

10.4.1 Weigh a suitable freeze-drier container to the nearest 0.1 g and record this weight as Wt.

10.4.2 Place the biomass material in the container. For solid samples, do not fill the container more than half full. For liquid or slurry materials, limit the sample to the amount of material that gives a uniform coating of around 0.5 cm on the walls of the container when the sample is frozen.

10.4.3 Weigh the container and biomass to the nearest 0.1 g and record this weight as Wi.

10.4.4 Combine the dry ice and acetone in a shallow container suitable for shell freezing.

10.4.5 Place the freeze dry flask containing the biomass sample in the dry ice acetone mixture. Slowly turn the container (10 r/min) to freeze the material into a uniform layer on the walls of the container.

10.4.6 Immediately place the container on the freeze-drier and allow the material to dry until all visible traces of ice and frost are gone from the sample. This process typically takes 12 h for small (<20 g) samples, and can extend to more than 96 h for large samples (>250 g).

10.4.7 Remove the container and biomass from the freeze drier.

10.4.8 Allow the sample to warm to room temperature.

10.4.9 Weigh the container and biomass to the nearest 0.1 g and record this weight as W_f.

10.4.10 Mill the dried biomass sample using one of the following procedures.

10.4.10.1 For small quantities (<20 g) containing material that would not pass through a 20 mesh screen, reduce the particle size of the solid residue by knife-milling the entire sample through an intermediate size knife-mill with a 1 mm screen. If the sample size is sufficient, sieve the sample as described in Method A, steps 10.2.5 through 10.2.14.

10.4.10.2 For larger quantities (>20 g) containing material that would not pass through a 20 mesh screen, reduce the particle size of the solids by knife-milling, sieving and riffling the entire sample as described in Method A steps 10.2.5 through 10.2.18.

10.4.11 Using the equations in Section 11, calculate the percent of each fraction in the original, biomass sample. The mass fraction is used to reconstruct the composition of the original biomass sample. In most cases, the composition of non-ash biomass in the fines can be assumed to be the same as the composition of the non-ash biomass in the –20/+80 fraction.

10.4.12 If the total sample needs to be subdivided into smaller samples, use the riffler to make this separation whenever possible.

10.4.13 If the prepared biomass sample is not analyzed immediately after sieving and riffling, the sample should be stored in an airtight container or sealable polyethylene bag and

kept at -20°C until needed.

11. Calculations

11.1 To calculate the fraction percent of –20/+80 mesh fraction, use the following equation:

$$Fraction_{20/80}\% = \left(\frac{(Wt_{20/80}}{(Wt_{20/80} + Wt_{80})} \right) \times 100$$

Where:

Wt $_{20/80}$ = weight of –20/+80 mesh fraction (g)
Wt_{80} = weight of fines fraction (g)

11.2 To calculate the fraction percent of -80 mesh fraction, use the following equation:

$$Fraction_{80}\% = \left(\frac{(Wt_{80}}{(Wt_{20/80} + Wt_{80})} \right) \times 100$$

Where:

Wt $_{20/80}$ = weight of –20/+80 mesh fraction (g)
Wt_{80} = weight of fines fraction (g)

11.3 To calculate the percent of total solids obtained by drying at 45ºC (Method B), use the following equation:

$$\%T_{45} = \left(\frac{(W_f - W_t)}{(W_i - W_t)} \right) \times 100$$

Where:

% T_{45} = percent total solids of a sample oven dried at 45ºC,
W_t = tare weight of freeze-drier container,
W_i = initial weight of container and sample
W_f = final weight of container and sample.

11.3.1 To calculate the percent of total solids obtained by freeze drying (Method C), use the following equation:

$$\%T_{fd} = \left(\frac{(W_f - W_t)}{(W_i - W_t)} \right) \times 100$$

Where:

% T_{fd} = percent total solids of a freeze-dried sample,
W_t = tare weight of freeze-drier container,
W_i = initial weight of container and sample
W_f = final weight of container and sample.

11.4 For larger biomass samples that must be prepared in batches, record the total percent solids of each fraction as well as the combined weight of the biomass in each fraction,

$(W_f - W_t)$.

11.5 To calculate the composition of constituent X in the original biomass sample, use the following equation:

$$\%X_{Origianl} = \left(\%X_{-20/+80} \times Fraction_{-20/+80}\%\right) + \left(\%X_{-20/+80} \times \left(\frac{100 - Fraction_{-80}\%}{100}\right)\right)$$

12. Report Format

12.1 Report weight percent prepared biomass and weight percent fines. Report results on an oven dry weight basis.

13. Precision and Bias

13.1 *Repeatability* – Duplicate determinations on splits of the gross sample, by the same operator, using the same sieves, should duplicate the percent mass fractions within 2% absolute.

13.2 *Reproducibility* - Duplicate determinations on splits of the gross sample, by different operators, using the same sieves, should duplicate the percent mass fractions within 2% absolute.

13.3 *Bias* - Since there is not an appropriate standard reference material, no statement about bias can be made.

13.4 *Round robin testing* – For a report documenting an international round robin test of biomass analysis methods, including this procedure, see Milne et al., 1992.

14. Quality Control (all items bellow, missing)

14.1 Reported Significant Figures or decimal places: Determined by data quality objectives and laboratory specific Quality Assurance Plan, see LAP "Rounding and Significant Figures".

14.2 Replicates: not applicable

14.3 Blank: not applicable

14.4 Relative percent difference criteria: not applicable

14.5 Calibration verification standard: not applicable

14.6 Sample size: not applicable

14.7 Sample storage: If the prepared biomass sample is not analyzed immediately after sieving and riffling, the sample should be stored in an air-tight container or sealable polyethylene bag and kept at –20ºC until needed.

14.8 Standard storage: not applicable

14.9 Standard preparation: not applicable

14.10 Definition of a batch: not applicable

14.11 Control charts: not applicable

15. Appendices

15.1 None

16. References

16.1 Moore, W., and D. Johnson. 1967. *Procedures for the Chemical Analysis of Wood and Wood Products*. Madison, WI: U.S. Forest Products Laboratory, U.S. Department of Agriculture

16.2 ASTM E 1757 - 01 "Standard Practice for Preparation of Biomass for Compositional

Analysis" In *2003 Annual Book of ASTM Standards, Volume 11.05*. Philadelphia, PA: American Society for Testing and Materials, International.

16.3 NREL BAT Team Laboratory Analytical Procedure "Standard Method for the Determination of Total Solids in Biomass."

16.4 TAPPI Test Method T264 cm-97, "Preparation of wood for chemical analysis" *In Tappi Test Methosd 2002-2003*. Atlanta, GA: Technical Association of the Pulp and Paper Industry

16.5 National Forage Testing Association Methods, available on-line at http://www.foragetesting.org/lab_procedures.php, Omaha, Nebraska, National Forage Testing Association, Accessed April 2004

16.6 Milne, T. A.; Chum, H. L.; Agblevor, F. A.; Johnson, D. K. (1992). "Standardized Analytical Methods" Biomass & Bioenergy. Proceedings of International Energy Agency Bioenergy Agreement Seminar", 2-3 April 1992, Edinburgh, U.K.. Vol. 2(1-6), 1992; pp. 341-366